Journal of Medical Virology 60:342–346 (2000)

Adenovirus Surveillance on Children Hospitalized for Acute Lower Respiratory Infections in Chile (1988–1996)

Carmen Larrañaga,[1]* Adriana Kajon,[2] Eliecer Villagra,[1] and Luis F. Avendaño[1]

[1]*Programa de Virología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile*

[2]*Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Argentina*

Adenoviruses (Ad) play an important role in the etiology of acute lower respiratory tract infections (ALRI) in young children in Chile. Our aim was to correlate the clinical severity of the infections with the Ad strains isolated during surveillance over 8 years. From 1988 through 1996, nasopharyngeal aspirates (NPA) were obtained for viral isolation and immunofluorescence assay (IFA) from children under 2 years of age hospitalized for ALRI; Ad isolates were further studied by restriction enzyme analysis of genomic DNA. Of 3,097 cases enrolled, the Ad isolation rate was 12.6%. The most common admission diagnoses among Ad-positive cases were pneumonia and wheezing bronchitis (69.8%). Duration of Ad shedding was studied in 74 cases by IFA. Children excreting Ad for 4 or more days had a longer hospital stay than those shedding for 1–3 days (mean: 16.8 and 7.2 days, respectively; $P < .01$). Viral shedding for more than 3 days was associated with more severe outcomes. Genome typing of 221 out of 390 Ad isolates resulted in 87 subgenus C and 134 subgenus B strains, including 123 Ad genome type 7h (55.6%, $P < .01$). The IFA from the NPA was more sensitive for the detection of subgenus B (51.5%) than subgenus C infections (24.1%, $P < .01$). Children shedding Ad 7h had longer hospital stays ($P < .01$), a higher frequency of rectal temperatures over 39°C ($P < .01$), and greater need for additional oxygen ($P < .02$) than subgenus C cases. Four cases requiring mechanical ventilation were associated with Ad 7h infections. The data presented show that, in children hospitalized for ALRI, the genome type 7h was associated with a more severe clinical outcome. ***J. Med. Virol. 60:342–346, 2000.***

KEY WORDS: adenovirus respiratory infections; infantile ALRI; adenovirus epidemiology; adenovirus in Chile

INTRODUCTION

Acute respiratory tract infections (ARI) are a major worldwide public health problem causing high morbidity and mortality rates in infants and children [P.A.H.O., 1985; Avendaño, 1997]. In Chile, ARI are the most common cause of outpatient visits and hospitalization in children, representing the leading cause of infant mortality in the post-neonatal period. ARI have a clear predominance during cold seasons, some of them being severe enough to require hospitalization [Kaempfer and Medina, 1992]. Respiratory syncytial virus (RSV) and adenoviruses (Ad) play a pivotal role in the etiology of acute lower respiratory tract infections (ALRI) requiring hospitalization. In Chile, during cold seasons, RSV and Ad are detected in 50–60% and 10–25% of cases hospitalized for ALRI respectively [Vicente et al., 1988; Avendaño et al., 1991]. Excluding winter, Ad clearly predominates over RSV as cause of ALRI [Avendaño et al., 1991; Papic et al., 1992].

Children with respiratory Ad infections may have a wide variety of presentations, ranging from a febrile flu-like syndrome to a fatal pneumonia [Wadell, 1987]. Viral or host-related risk factors for severe diseases have not been clearly established. The evidence of a virus-related risk factor is supported by the occurrence of severe adenoviral epidemics and nosocomial outbreaks, which suggests the emergence of particularly virulent Ad strains. In Chile, we have observed nosocomial and day care center Ad outbreaks [Kajon and Vicente, 1990; Wu et al., 1990]. Certain serotypes, v gr.

Grant sponsor: FONDECYT; Grant number: 91-1133; Grant sponsor: Universidad de Chile; Grant number: M-3182-90-12.

*Correspondence to: Dr. Carmen Larrañaga, Programa de Virología, Facultad de Medicina, Universidad de Chile, Casilla 9183, Correo Central, Santiago, Chile.
E-mail: lavendan@machi.med.uchile.cl

Accepted 15 June 1999

3,7,21 have been associated with more severe ALRI in infants [Straube et al., 1983; Wadell, 1987] and Ad genome typing with restriction enzymes has recently shown a relationship between viral strain and clinical severity [Adrian et al., 1986; Kajon et al., 1994].

In this study, we present the molecular epidemiology and clinical findings of adenoviral infection obtained during an 8-year surveillance of ALRI in Santiago, Chile.

MATERIALS AND METHODS

Population

A total of 3,097 of 9,919 children younger than 2 years of age, admitted to the Roberto del Río Children's Hospital for ALRI, were studied prospectively for respiratory viruses detection and clinical outcome from May 1988 to December 1996. Due to the seasonality of the hospitalization, during cold season we randomly selected one of three admissions for ALRI, but during warm season we enrolled almost all of them. Exclusion criteria included children with history of prematurity, underlying pulmonary, cardiac or neurological diseases, and/or children who required previous hospitalization for any reason.

Clinical Severity Assessment

For each patient, the age, sex, diagnosis on admission, personal or family history of asthma, etc., were obtained from the hospital charts. Clinical severity was assessed for each child according to predefined parameters: total days of hospitalization; total days with supplemental oxygen, bronchodilators, intravenous steroids, and/or mechanical ventilation; and mortality.

Diagnosis of Respiratory Viruses

Specimen collection. A nasopharyngeal aspirate (NPA) was obtained from each child within the first 48 hr after admission; a second NPA was obtained 24–48 hr later. The samples were collected using a plastic catheter with specimen trap and transported within 1 hr on wet ice to the laboratory. The admission sample was processed for viral detection by isolation in cell culture and indirect immunofluorescence assay (IFA). Sample 2 was tested only for IFA. Duration of Ad shedding was studied in a subset of 74 children who had an Ad-positive IFA on admission, by daily NPA sampling until two sequential negative IFA were obtained; all of these cases had one or more NPA-positive specimens for Ad by cell culture isolation.

Viral isolation. Each specimen was processed as described elsewhere [Avendaño et al., 1991]. Briefly, the samples were inoculated into HEp-2 cells and observed every other day for cytopathic effect (CPE). The presence of viruses was confirmed by IFA on all tubes showing CPE, as well as on tubes without such effect, at the end of the 10 days observation period [Ballew et al., 1984].

IFA. For indirect detection of viral antigen, smears were prepared in triplicate and fixed with cold acetone. IFA was run for RSV, Ad, influenza (I) A-B, parainfluenza (PI) 1–3. Standard indirect immunofluorescent staining was done as described elsewhere [Ballew et al., 1984; Larrañaga et al., 1990; Avendaño et al., 1991] using monoclonal antibodies for RSV, Ad, PI 1, and PI 2, kindly provided by Dr. L. Anderson (CDC, Atlanta, GA), and for PI 3 and IA-B provided by Dr. P. Pothier (Dijon, France). Rabbit polyclonal antibodies for Ad were supplied by Dr. G. Wadell (Sweden). We used commercial anti-mouse and anti-rabbit antibodies conjugated to fluorescein (Sigma). Specimens were considered positive by IFA if the smear showed greater than 2+ particulate green fluorescence in three or more cells.

Ad genome type analysis. Ad isolates were propagated on HEp-2 or A-549 cells. Viral DNA was extracted as described by Shinagawa et al. [1983] and studied further with different endonucleases [Kajon et al., 1994; Kajon and Wadell, 1994]. Isolates belonging to subgenus B were typed by restriction enzyme analysis with the endonucleases BamHI and SmaI, and for further characterization, Xhol. For subgenus C isolates, the endonucleases used were BamHI, SmaI, BglII, and HindIII. The profiles were compared with those published for the prototypes and for more recently circulating genomic variants [Adrian et al., 1986; Wadell, 1987; Kajon et al., 1993, 1994; Kajon and Wadell, 1994]. Genome types obtained were matched with clinical information.

Statistical Analysis

Information related to clinical as well as viral results were analyzed with EpiInfo, version 5. Statistical comparisons were performed using chi square or Fisher's exact test. Comparisons of continuous variables with abnormal distribution, were done by using chi square test for the elaborate median.

RESULTS

Population

Viral infections were detected in 1,279 of 3,097 ALRI cases analyzed (41.3%), RSV being the agent most frequently detected (813 cases: 26.3%). Ad was detected on admission in 390 cases (12.6%), in 265 (8.6%) as a single viral infection. Mixed viral infections that included Ad were detected in 125 cases (4.0%): 106 with RSV, 12 with a parainfluenza virus, 4 with an influenza virus, and 3 cases with Ad and RSV plus parainfluenza or influenza viruses.

The annual distribution of Ad infections was not uniform, ranging from 4.5% to 28.8% (mean 12.6%). The highest Ad detection rate was observed between 1988 and 1992 (13.6–28.8%) and the rate declined significantly between 1993 and 1996 (4.5–7.5%) ($P < .01$). In contrast to RSV, Ad was found year-round, without a particular seasonality (Fig. 1).

The most frequent admission diagnoses for Ad respiratory infection were pneumonia and wheezing bronchitis (69.8%). Children between 0 and 5 months of age were the most common age group affected (41.6%). Males represented the 63.8% of positive cases (Table I).

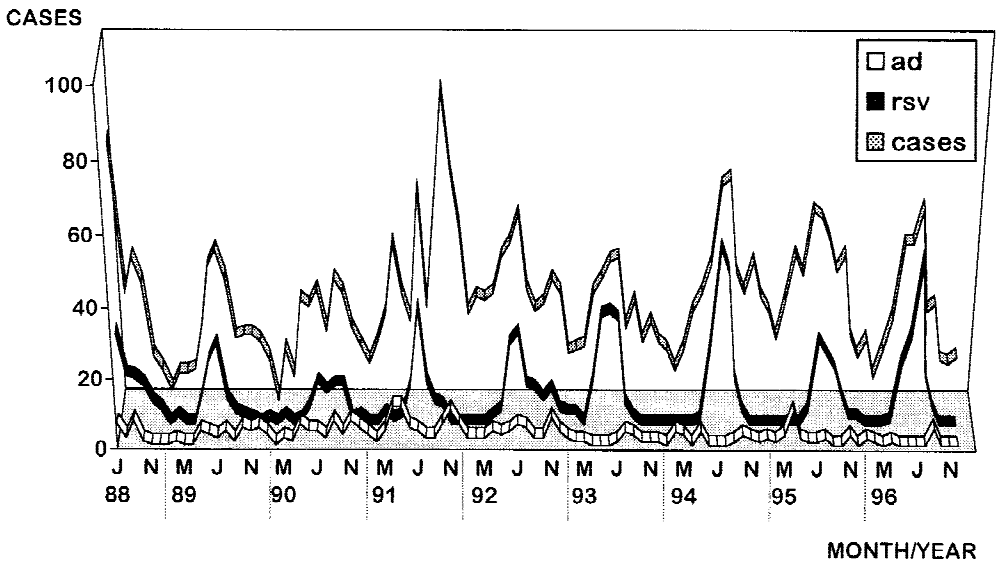

Fig. 1. Seasonal and year distribution of respiratory syncytial virus (RSV) and adenovirus infections in children younger than 2 years of age hospitalized for acute lower tract respiratory infections (Santiago, June 1988 to December 1996).

TABLE I. Characteristics of Infants and Children Younger Than 2 Years of Age Hospitalized for Acute Lower Adenovirus Respiratory Infections (Santiago, June 1988 to December 1996)

Factor	%
Clinical diagnosis	
Pneumonia + wheezing bronchitis	69.8
Pneumonia	23.1
Wheezing bronchitis	3.0
Bronchitis	2.0
Bronchiolitis	1.5
Pleuropneumonia	0.5
Age (months)	
0–5	41.6
6–11	38.9
12–23	19.5
Sex	
Male	63.8
Female	36.2

Duration of Ad Shedding

Among 74 Ad-positive cases from which daily NPA specimens were collected until the viral excretion became negative, shedding ranged from 1 to 17 days. Thirty-five cases (47.3%) excreted virus for 3 days or less and 39 patients (52.7%) for 4 or more days, including 18 children who shed for more than 7 days. Children shedding Ad for 1–3 days did not differ from those excreting for 4 or more days in age, sex, or clinical signs and symptoms upon admission. Hospital stay was longer in patients with more prolonged Ad shedding (Fig. 2). For 35 infants who excreted Ad for 1–3 days the mean hospital stay was 7.2 days (range 3–17), as compared with 16.8 days (range 5–70) for 21 children excreting Ad for 4–6 days ($P < .01$); in the 18 patients who shed Ad during 7 or more days the mean hospital stay was 21.5 days (range 10–41). The relationship between duration of viral shedding and clinical severity is shown in Table II. Viral excretion for more than 3 days was significantly associated with more frequent requirement of oxygen (76.9%), aerosolized bronchodilators (87.2%), and steroids (38.5%). Mechanical ventilation was used in 1 case excreting virus for 2 days and in 3 patients who had Ad shedding between 4 and 9 days.

Ad Genome Typing

To characterize the isolates obtained from the clinical cases aforementioned, we used restriction enzyme analysis. Genome typing of 221/390 Ad isolates identified 134 strains belonging to Ad subgenus B: 123 genome type 7h (55.6%), ($P < .01$), 8 type 3, 2 type 11, and 1 type 16. Eighty-seven strains corresponded to subgenus C: 58 type 2, 21 type 1, and 8 type 5. No differences were observed in sex, age, and clinical features at admission, comparing subgenus B and C cases.

Inmunofluorescence tests allowed the rapid diagnosis of Ad infection in 90 of the 221 cases (40.7%). The subgenus B was more readily detectable by IFA than subgenus C (69/134 = 51.5% vs. 21/87 = 24.1%, respectively, $P < .01$). The duration of shedding for over 3 days was more commonly observed in subgenus B patients (26/69; 37.7%) than in those infected with subgenus C (3/21; 16.6%, $P < .05$%).

A comparison of clinical parameters showed that a more severe disease was associated with Ad subgenus B than C. Children shedding Ad 7h had significantly longer hospital stays (mean 11 days, median 7.5 days, range 2–120 days, SD 13.6 days) than patients excreting Ad subgenus C (mean 7 days, median 5 days, range 1–42 days, SD 7.5 days) ($P < .01$). Also 37/110 (33.6%) of Ad 7h cases required an hospitalization for more than 10 days, as compared with 9/72 (12.5%) of subgenus C cases ($P < .01$).

The need for supplemental oxygen was significantly

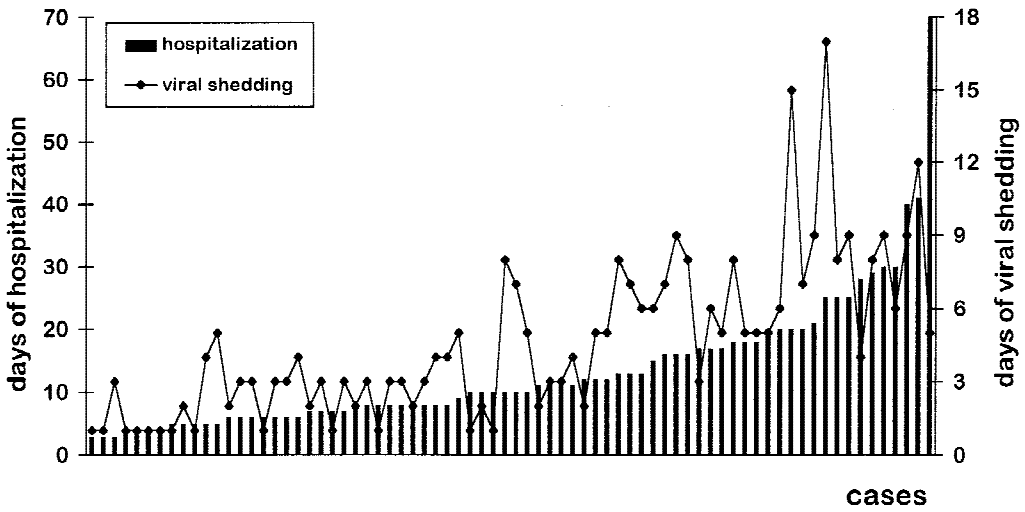

Fig. 2. Viral shedding and duration of hospitalization of 74 children younger than 2 years of age hospitalized for adenovirus acute lower respiratory tract infections (Santiago, June 1988 to December 1996).

TABLE II. Duration of Viral Shedding and Clinical Management of 74 Children Younger Than 2 Years of Age With Acute Lower Adenovirus Respiratory Infections (Santiago, June 1988 to December 1996)

	Duration of viral shedding			
Therapy	0–3 days (n = 35)	≥4 days (n = 39)	Total (n = 74)	P <
Oxygen	11	30	41	.01
Aerolized Salbutamol	19	34	53	.01
Steroids	1	15	16	.01
Ventilatory Assistance	1	3	4	NS[a]

[a]NS, not significant.

TABLE III. Genome Types of Adenovirus and Clinical Findings of Children Younger Than 2 Years of Age Hospitalized for Acute Lower Respiratory Infection (Santiago, June 1988 to December 1996)

Genome types	Oxygen n (%)	T ≥ 38°C n (%)	T ≥ 39°C n (%)
B7h (n = 110)	51 (46.3)	76 (69.1)	54 (49.1)
C1 (n = 20)	6 (30.0)	10 (50.0)	4 (20.0)
C2 (n = 52)	13 (25.0)	33 (63.5)	14 (26.9)
P <	.02	NS[a]	.01

[a]NS, not significant.

higher in cases shedding Ad 7h (46.3%) than in those shedding Ad serotypes 1 and 2 (25% and 30%, respectively, $P < .02$). Furthermore, patients infected by Ad 7h had rectal temperatures over 39°C (49.1%) more frequently than those patients infected with Ad serotypes 1 and 2 (20% and 26.9%, respectively, $P < .01$, Table III). The four cases requiring mechanical ventilation were associated with Ad 7h strain.

DISCUSSION

This study has confirmed that Ad are the second most frequent cause of severe viral pneumonia in infants and young children in Chile, as in other countries of Latin America [Videla et al., 1998]. This study also shows that whereas RSV infections occur every year as epidemics during cold seasons, Ad infections are detected during the whole year.

Two characteristics of Ad infection must be emphasized. First, its capacity to cause severe infections with long-term sequelae, which can be fatal in otherwise healthy infants. Secondly, Ad infections have the potential of generating outbreaks in closed environments, i.e., nosocomial infections. The clinical characteristics of ALRI on admission, as well as hemogram and X-rays, do not allow differentiation of viral or bacterial etiology. Persistent high fever, progressive pulmonary signs, and general malaise observed during Ad infections imitate severe bacterial infections, and the patients are treated unsuccessfully with multiple antibiotics [Straube et al., 1983; Wadell, 1987; Wu et al., 1990]. In these refractory cases, Ad-specific etiology should be explored [Larrañaga et al., 1990; Ballew et al., 1984]. It has been demonstrated that rapid diagnostic techniques, such as IFA, are less sensitive than isolation for Ad detection, and unfortunately not many laboratories are able to isolate viruses, a drawback that represents a limiting factor for Ad diagnosis.

We postulated that the duration of Ad excretion could correlate with the severity of the infection, because severe infections should shed larger amounts of viruses for longer periods than mild ones. On the other side, a relevant problem in South America is that hospitals have multi crib-wards and high occupational rates, which facilitate nosocomial transmission. Therefore, the measurement of the duration of virus excretion by IFA, despite not being a very sensitive test, could help to formulate a case clinical prognosis and to reinforce measures to limit nosocomial spreads. Our viral shedding follow up in 74 cases from admission demonstrated a relationship between excretion over 4 days and higher clinical severity. This relationship was

most significant in those patients shedding Ad for more than 7 days. Furthermore, the predominant strain Ad 7h was more easily detected by IFA than Ad belonging to subgenus C. Because Ad isolation and typing are not techniques available in common hospital laboratories, we recommend performing serial IFA to improve the detection of Ad respiratory infections.

To analyze the risk factors associated with virus strain, we used genome typing. Previous reports had shown that the new genomic variant 7h had become predominant in the Southern cone of America in the past decade, being associated with more severe clinical presentations and fatal cases [Liq and Wadell, 1986; Kajon et al., 1990, 1994, 1996; Wu et al., 1990; Murtag et al., 1993; Kajon and Wadell, 1994]. In this study the 7h strain was predominant in the 221 cases enrolled and it was also associated with the more severe cases.

There were no fatal cases in this study, in which high-risk patients with underlying chronic diseases were not enrolled. The long-term pulmonary sequelae are under study in order to match clinical outcome with the adenoviral genome type.

The implementation of restriction enzyme genome analysis and neutralization assay for identification of Ad strain is currently restricted to a few research laboratories. The Ad genome type seems to be an important risk factor for severe infection and efforts should be made to develop rapid methods for strain analysis, i.e., polymerase chain reaction and monoclonal immune diagnoses. We believe that the measurement of viral shedding by IFA can be useful in the clinical and epidemiological management of respiratory Ad infections.

ACKNOWLEDGMENTS

We thank Inés Orellana and María Inés Espinoza for their excellent technical assistance.

REFERENCES

Adrian Th, Wadell G, Hierholzer J, Wignad R. 1986. DNA restriction analysis of Adenovirus prototypes 1 to 41. Arch Virol 91:277–290.

Avendaño LF. 1997. Resfrío común, influenza y otras infecciones respiratorias víricas. In: Meneghello J, editor. Pediatría. 5th ed. Buenos Aires, Argentina: Editorial Médica Panamericana. p 1264–1268.

Avendaño LF, Larrañaga C, Palomino MA, et al. 1991. Community and hospital acquired respiratory syncytial virus infections in Chile. Pediatr Infect Dis J 10:564–568.

Ballew H, Lyerla HC, Forrestier FT. 1984. Laboratory methods for diagnosing respiratory virus infections. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Kaempfer AM, Medina E. 1992. Hospitalización infantil en Chile: situación actual y perspectivas. Revista Chilena de Pediatría 63: 110–117.

Kajon A, Larrañaga C, Suárez M, Wadell G, Avendaño LF. 1994. Genome type analysis of Chilean adenovirus strains isolated in a children's hospital between 1988 and 1990. J Med Virol 42:16–21.

Kajon A, Mistchenko A, Videla C, Hortal M, Wadell G, Avendaño LF. 1996. Molecular epidemiology of adenovirus acute lower respiratory infections in children in the South Cone of South America (1991–1994). J Med Virol 48:151–156.

Kajon A, Vicente M. 1990. Molecular epidemiology of adenovirus isolated from hospitalized children with severe lower acute respiratory infection in Santiago, Chile. J Med Virol 30:294–297.

Kajon A, Vicente M, Avendaño LF, Hortal M, Wadell G. 1993. Genome types analysis of South American adenoviruses of subgenus C collected over a 7-year period. Arch Virol 132:29–35.

Kajon A, Wadell G. 1994. Genome analysis of South American adenovirus strains of serotype 7 collected over a 7-year period. J Clin Microbiol 32:2321–2323.

Larrañaga C, Avendaño LF, Gaggero A, et al. 1990. Diagnóstico de infección por Adenovirus y Virus Sincicial Respiratorio en lactantes. Revista Chilena de Infectología 7:67–171.

Liq G, Wadell G. 1986. Analysis of 15 different genome types of adenovirus type 7 on five continents. J Virol 60:331–335.

Murtag P, Cerqueiro C, Halac A, Avila M, Kajon A. 1993. Adenovirus type 7h respiratory infections: a report of 29 cases of acute lower respiratory diseases. Acta Paediatr 82:557–561.

P.A.H.O. Organización Panamericana de la Salud. 1985. Infecciones Respiratorias Agudas en los niños. Publicación Científica No. 493. Washington DC: P.A.H.O.

Papic Z, Rodríguez L, Larrañaga C, Avendaño LF. 1992. Virus respiratorios en lactantes con infecciones respiratorias altas y bajas. Revista Chilena de Pediatría 63:256–261.

Shinagawa M, Matsuda A, Ishiyama T, Goto H, Sato G. 1983. A rapid and simple method for preparation of adenovirus DNA from infected cells. Microbiol Immunol 27:817–822.

Straube RC, Thomson MA, Van Dyke RB, et al. 1983. Adenovirus type 7b in a children's hospital. J Infect Dis 147:16.

Vicente M, Wu E, Carrasco L, et al. 1988. Participación viral en las infecciones respiratorias agudas bajas del lactante. Revista Chilena de Pediatría 59:353–357.

Videla C, Carballal G, Misirlian A, Aguilar M. 1998. Acute lower respiratory infections due to respiratory syncytial virus and adenovirus among hospitalized children from Argentina. Clin Diagn Virol 10:17–23.

Wadell G. Adenoviruses. 1987. In: Zuckerman AJ, Banatvala JE, Pattison JR, editors. Principles and practice of clinical virology. London: John Wiley & Sons.

Wu E, Martínez V, Alvarez A, Larrañaga C, Vela H. 1990. Casos fatales de infección por Adenovirus. Revista Chilena de Pediatría 61:177–184.